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August 13, 2002

U.S. Environmental Protection Agency
Office of Pollution Prevention & Toxics
1200 Pennsylvania Avenue, NW
Mail Code 7401M
Washington, DC 20460

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Ladies and Gentlemen:

Summaries on a number of chemicals became available from the Japanese Ministry of Environment. These are just summaries, with limited information. No test methods descriptions or full reports are available.

Eastman Chemical Company is submitting portions of the summaries that describe results for a number of chemicals that are of interest to Eastman Chemical Company. We are providing this for your information, since there is not enough data available to fully evaluate the reported findings.

If you have questions, you may contact me by telephone at (423) 229-1654.

Very truly yours,

Karen R. Miller

Karen R. Miller
Technical Associate
Product Safety & Stewardship

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Results of Hazard Assessment on the Human Health Effect (Mammals)

1. Di-2-ethylhexyl phthalate

(1) Single-generation study with rodents

1) Changes in the maximum dose group (100 mg/kg/day)

A high liver weight value (absolute and relative) and swelling of the centrilobular hepatic cells were observed in F_0 dams. These changes have been previously described in the reliable report.

Low litter size values, decrease in the number of live offspring and the uterus weight and infertility in F_0 dams as well as atrophy or aplasia of the testis and epididymis and the bleeding from the testis in F_1 males which have been previously described were not observed in this study.

2) Changes in the low dose groups (10, 50, 250 and 1,250 μ g/kg/day)

An increase in the serum FSH concentration observed in F_1 females of the 50 μ g/kg/day dose group was considered to be within the range of physiological changes, because no effect on the ovary weight (absolute and relative), uterus weight (absolute and relative), histopathological findings, vaginal opening time, estrous cycle, mating results or the number of corpus luteum of pregnancy was observed. The serum FSH concentration was retested, but no significant difference was observed.

(2) *In vitro* studies

A human estrogen receptor (ER α and ER β)-competitive binding assay, E-screen assay using a human breast cancer cell line, androgen receptor-reporter gene assay using a human breast cancer cell line (agonist and antagonist), rat androgen receptor-competitive binding assay and yeast assay using human thyroid hormone receptors (TR α and TR β) were conducted.

As the results, the human estrogen receptor (ER α and ER β)-competitive binding assay showed this substance with weak activity, while the rat androgen receptor-competitive binding assay showed very weak activity. No significant activity was shown in the other studies.

(3) Hazard assessment at present and future courses of action

As described above, the general toxicity of di-2-ethylhexyl phthalate was observed only at a maximum dose (a concentration showing the effect in the previous report) in this study.

A further risk assessment of this substance with respect to human health is currently in progress with particular emphasis on general toxicity, and the assessment will be also conducted based on these study results.

3. Diethyl phthalate

(1) Single-generation study with rodents

1) Changes in the maximum dose group (2000 mg/kg/day)

Low body weight values, decrease in body weight gain, food consumption, the weight (absolute and relative) of the pituitary gland and thyroid gland, and the eosinophilic granular degeneration of liver were observed in F_0 dams. Low survival rate values and the number of live offspring were observed in F_1 offspring. Low body weight values, decrease in body weigh gain, sperm motility (motility rate of sperm) and the weight (absolute and relative) of the thymus gland and testis, delays in behavioral development (negative geotaxis), physical development (pinna unfolding and eyelid opening) and preputial separation, a decrease in the number of spermatogonia in the testis, a low or high blood FSH concentration values, and the local atrophy of seminiferous tubules in the testis were observed in F_1 males. Low body weight values, decrease in body weight gain, the weight (absolute and relative) of the thymus gland and kidney and the locomotor activity (horizontal activity and rearing), a high value of the anogenital distance (AGD) length (absolute and relative), delays in behavioral development (cliff drop avoidance response and negative geotaxis) and physical development (pinna unfolding and eyelid opening) were observed in F_1 females.

Low body weight values, decrease in body weight gain and the weight of the pituitary gland, a high liver weight value and eosinophilic granular degeneration of liver in F_0 dams, low survival rate values and the number of live offspring in F_1 offspring, low body weight values and sperm motility (motility rate of sperm) in F_1 males and a low body weight value in F_1 females have previously been described in the reliable report.

A low liver weight value in F_0 dams and supernumerary rib in the fetuses described in the previous report were not observed in this study.

2) Changes in the low dose groups (0.4, 2, 10 and 50 μ g/kg/day)

A low value for the weight (absolute and relative) of the pituitary gland was observed in F_0 dams of the 0.4, 2, 10 and 50 μ g/kg/day dose groups, but this change was considered to be within the range of physiological changes, since no adverse effect on the pregnancy maintenance, parturition, nursing, developmental differentiation of the offspring and reproductive capability probably caused by the alteration in pituitary function was observed. Behavioral development (negative geotaxis) tended to be delayed with a dose-response relationship, and the locomotor activity tended to fall with the dose-response relationship in F_1 females although no significant difference was observed.

A low value for the weight (absolute and relative) of the thyroid gland was observed in F_0 dams of the 10 and 50 μ g/kg/day dose groups, but this change was considered to be within the range of

physiological changes, since no adverse effect on the pregnancy maintenance, parturition, nursing, developmental differentiation of the offspring and reproductive capability probably caused by the alteration in thyroid function was observed.

A delay of preputial separation was observed in F_1 males (after weaning) of the 50 μ g/kg/day dose group, but this change was considered to be within the range of physiological changes, since no change in the weight of the accessory reproductive organs and sperm test results was observed.

(2) *In vitro* studies

A human estrogen receptor (ER α and ER β)-competitive binding assay, E-screen assay using a human breast cancer cell line, androgen receptor-reporter gene assay using a human breast cancer cell line (agonist and antagonist), rat androgen receptor-competitive binding assay and yeast assay using human thyroid hormone receptors (TR α and TR β) were conducted.

As the results, the human estrogen receptor (ER α and ER β)-competitive binding assay and E-screen assay using a human breast cancer cell line showed very weak activity. The androgen receptor-reporter gene assay using a human breast cancer cell line (antagonist) and the rat androgen receptor-competitive binding assay provided the IC_{50} values of 7.9×10^{-5} M and 1.5×10^{-3} M, respectively. No significant activity was shown in the androgen receptor-reporter gene assay using a human breast cancer cell line (agonist) and yeast assay using human thyroid hormone receptors (TR α and TR β).

(3) Results of the *in vivo* studies conducted by the Ministry of Economy, Trade and Industry

A uterine proliferation assay (estrogenic activity and anti-estrogenic activity) and Hershberger assay (androgenic activity and anti-androgenic activity) were conducted with this substance at doses of 200-2,000 mg/kg/day.

Negative results were obtained in all of these studies.

(4) Hazard assessment at present and future courses of action

As described above, the general toxicity of diethyl phthalate was observed only at the maximum dose (the concentration showing the effect in the previous report) in this study.

These study results will be used for the initial environmental risk assessment of this substance in the future.

5. Di-2-ethylhexyl adipate

(1) Single-generation study with rodents

1) Changes in the maximum dose group (600 mg/kg/day)

A high liver weight value (absolute and relative) was observed in F_0 dams and a high value for the number of stillborn infants and a low value of the weaning rate were observed in F_1 offspring. A low value for the blood testosterone concentration and decreases in the rate of total abnormal spermatozoa and the rate of spermatozoa with abnormal head were observed in F_1 males and a low value for the amount of ER α mRNA induced in the uterus was observed in F_1 females.

A high liver weight value in F_0 dams and high value for the number of stillborn infants in F_1 offspring have been previously described in the reliable report.

An increase in fetal malformation described in the previous report was not observed in this study.

2) Changes in the low dose groups (15, 150, 1,500 and 15,000 μ g/kg/day)

A low value for body weight gain was observed in F_0 dams (11 days after parturition) of the 150 μ g/kg/day dose group, however it was considered to be within the range of physiological changes, since there was no significant difference in the body weight gain after 14 days of parturition.

A low value for the blood testosterone concentration was observed in F_1 males of the 1,500 and 15,000 μ g/kg/day dose groups, but it was considered to be within the range of physiological changes, since there was no change in the weight of reproductive organs and reproductive capability.

(2) *In vitro* studies

A human estrogen receptor (ER α and ER β)-competitive binding assay, E-screen assay using a human breast cancer cell line, androgen receptor-reporter gene assay using a human breast cancer cell line (agonist and antagonist), rat androgen receptor-competitive binding assay and yeast assay using human thyroid hormone receptors (TR α and TR β) were conducted.

As the results, the human estrogen receptor (ER α and ER β)-competitive binding assay showed very weak activity of this substance. The rat androgen receptor-competitive binding assay showed weak activity. No significant activity was shown in the E-screen assay using a human breast cancer cell line, androgen receptor-reporter gene assay using a human breast cancer cell line (agonist and antagonist) and yeast assay using human thyroid hormone receptors (TR α and TR β).

(3) Hazard assessment at present and future courses of action

As described above, the general toxicity of di-2-ethylhexyl phthalate was observed only at the maximum dose (the concentration showing the effect in the previous report) in this study.

The initial environmental risk assessment of this substance is currently in progress, and the assessment based on these study results will be also conducted.

10. Di-n-butyl phthalate

(1) Single-generation study with rodents

1) Changes in the maximum dose group (250 mg/kg/day)

A low survival rate value was observed in F_1 offspring and low values for the AGD length (absolute and relative) and the amount of ER α mRNA induced in the prostate gland, and the absence, hypoplasia and atrophy of the reproductive organ and accessory reproductive organ were observed in F_1 males. A low value for the amount of ER α mRNA induced in the prostate gland was observed in F_1 males (70 days after birth), and low body weight values, the weight (absolute and relative) of the prostate gland and thyroid gland, the amount of ER α mRNA induced in the uterine and the number of live fetuses and a delay of vaginal opening were observed in F_1 females.

The low value of AGD length and the absence, hypoplasia and atrophy of the reproductive organ and accessory reproductive organ in F_1 males have been previously described in the reliable report.

2) Changes in the low dose groups (31, 63, 125, 250 and 500 μ g/kg/day)

A high value for the weight (absolute and relative) of the dorsal prostate gland, a delay of preputial inversion and a low value for the amount of ER α mRNA induced in the prostate gland were observed in F_1 males of the 31 μ g/kg/day dose group. A low value for the weight (absolute and relative) of the pituitary gland and a high value for the weight (absolute and relative) of the dorsal prostate gland were observed in F_1 males of the 63 μ g/kg/day dose group. High values for the weight (absolute and relative) of the thyroid gland, epididymis, dorsal prostate gland and penis were observed in F_1 males of the 250 μ g/kg/day dose group. A low value for the weight (absolute and relative) of the pituitary gland and a low value for the amount of ER α mRNA induced in the prostate gland were observed in F_1 males of the 500 μ g/kg/day dose group. Early vaginal opening and a low body weight value were observed in F_1 females of the 31 μ g/kg/day dose group. A low value for the weight (absolute and relative) of thyroid gland was observed in F_1 females of the 125 and 250 μ g/kg/day dose groups and early vaginal opening was observed in F_1 females of the 500 μ g/kg/day dose group.

(2) *In vitro* studies

A human estrogen receptor (ER α and ER β)-competitive binding assay, E-screen assay using a human breast cancer cell line, androgen receptor-reporter gene assay using a human breast cancer cell line (agonist and antagonist), rat androgen receptor-competitive binding assay and yeast assay using human thyroid hormone receptors (TR α and TR β) were conducted.

As the results, the human estrogen receptor (ER α and ER β)-competitive binding assay and the rat androgen receptor-competitive binding assay showed weak activity. No significant activity was shown in the other studies.

(3) Results of the *in vivo* studies conducted by the Ministry of Economy, Trade and Indsutry

A uterine proliferation assay was conducted with this substance at a dose of 40-1,000 mg/kg/day. Negative results were obtained in this assay.

(4) Future courses of action

The additional single-generation study with rodents is currently in progress. The overall hazard assessment will be conducted based on the additional study result, the result of the on-going study conducted by the Ministry of Economy, Trade and Industry and the reliable literature information.

11. Summary

The results of the "single-generation studies with rodents" and the "*in vitro* studies" of ten priority substances in fiscal 2000 were summarized.

As the results, no obvious endocrine disrupting activity was observed in any of these substances (the additional study of di-n-butyl phthalate is now in progress) at a low dose (the range of concentrations to which human is probably exposed).

With respect to the some substances, the changes that could not be definitely confirmed as endocrine disrupting activity though a significant difference was observed were to be studied in the future since it was difficult to demonstrate the significance at the present.

The effect considered to represent general toxicity was observed at the maximum dose (the concentration showing the effect in the previous reports).

These study results will be used for the initial environmental risk assessment of these substances.

Results of hazard assessment on the ecological effect (fish)

2. Di-n-butyl phthalate

(1) Study with medaka

1) Vitellogenin assay

No statistically significant change in the vitellogenin concentration in the liver was observed in both sexes of the 24.4, 55.3, 133, 328 and 822 μg/L (actual measured concentrations) groups. A high value with statistical significance for the hepatosomatic index was observed in males of the groups exposed at a concentration of 328 μg/L or more.

2) Partial life cycle test

This test was conducted with this substance at an exposure concentration of 7.09, 21.9, 72.8, 235 and 850 μg/L (actual measured concentrations). A low value with statistical significance for the hatchability was observed in the 850 μg/L group, and a high value with statistical significance for the mortality was observed in the groups exposed at a concentration of 72.8 μg/L or more. All of the fish in the 850 μg/L group died. No change in the total length, body weight, vitellogenin, concentration in the liver or the gonad index was observed in the groups exposed at a concentration of 235 μg/L or lower.

The histological test of the genital gland revealed a few fish having testicular eggs in the 7.09 and 72.8 μg/L groups. A statistically significant difference in the incidence of testicular eggs was observed in the 72.8 μg/L group.

A high value with statistical significance for the hepatosomatic index was observed in females of the 7.09 μg/L group.

3) Full life cycle test

This test was conducted with this substance at an exposure concentration of 2.61, 7.52, 23.9, 74.5 and 233 μg/L (actual measured concentrations). However, no statistically significant change in the hatchability, the number of days until hatching, mortality, total length, body weight, the vitellogenin concentration in the liver, the number of eggs laid, fertility and hepatosomatic index in the F_0 generation or mortality in the F_1 generation was observed.

The histological test of the genital gland in the F_0 generation revealed a few fish having testicular eggs in the 2.61, 74.5 and 233 μg/L groups. A statistically significant difference in the incidence of testicular eggs was observed in the 233 μg/L group, and a high value with statistical significance

for the gonad index was observed in males of the 23.9 μ g/L group.

In the F_1 generation, high values with statistical significance for the hatchability, total length and body weight were observed in the 7.52 μ g/L dose group. A statistically significant increase in the number of days until hatching was observed in the 74.5 μ g/L dose group and a high value with statistical significance for the hatchability and a statistically significant increase in the number of days until hatching were observed in the 233 μ g/L dose group.

A high value with statistical significance for the vitellogenin concentration in the liver was observed in males of the 2.61, 7.52 and 74.5 μ g/L groups.

A histological test of the genital gland revealed a few fish having testicular eggs in the 2.61, 7.52, 23.9 and 74.5 μ g/L dose groups. A statistically significant difference in the incidence of testicular eggs was observed in the 7.52 μ g/L group.

(2) *In vitro* study

A medaka estrogen receptor (ER α and ER β)-competitive binding assay, medaka estrogen receptor-reporter gene assay (ER α and ER β) and medaka androgen receptor-reporter gene assay were conducted.

As a result, the medaka estrogen receptor (ER α and ER β)-competitive binding assay showed that the relative binding strength with respect to the estrogen receptor was about 1/4, 350 (ER α) and 1/15, 870 (ER β) compared with estradiol. The medaka estrogen receptor-reporter gene assay (ER α) showed the activity with respect to ER α, but the IC_{50} value was not obtained. No statistically significant activity was shown in the medaka estrogen receptor-reporter gene assay (ER β) and medaka androgen receptor-reporter gene assay.

(3) Future courses of action

As described above, the general toxicity (high values with statistical significance for the hepatosomatic index, mortality and the gonad index, a significantly low value of hatchability and a statistically significant increase in the number of days until hatching) was observed with di-n-butyl phthalate at a relatively high concentration in this study. With respect to testicular eggs observed at a low frequency without a dose-response relationship and a high value with statistical significance for the vitellogenin concentrations in liver, further study on the effect on population etc. should be conducted in the future.

3. Di-2-ethylhexyl phthalate

(1) Study with medaka

1) Vitellogenin assay

No statistically significant effect of this substance on the vitellogenin concentration in the liver and hepatosomatic index was observed in males exposed to this substance at a concentration of 19, 43, 96, 210 and 410 μ g/L (actual measured concentrations).

2) Partial life cycle test

This test was conducted with this substance at an exposure concentration of 11.0, 28.4, 73.4, 186 and 446 μ g/L (actual measured concentrations). No statistically significant change in the hatchability, the number of days until hatching, mortality, total length, body weight, vitellogenin, concentration in the liver, or the gonad index and hepatosomatic index was observed.

The histological test of the genital gland revealed a few fish having testicular eggs in the 73.4 μ g/L group.

(2) *In vitro* study

A medaka estrogen receptor (ER α and ER β)-competitive binding assay, medaka estrogen receptor-reporter gene assay (ER α and ER β) and medaka androgen receptor-reporter gene assay were conducted.

As a result, the medaka estrogen receptor-competitive binding assay (ER β) showed that the relative binding strength with respect to the estrogen receptor was about 1/270 compared with that of estradiol. No statistically significant activity was shown in the medaka estrogen receptor-competitive binding assay (ER α), medaka estrogen receptor-reporter gene assay (ER α and ER β) and medaka androgen receptor-reporter gene assay.

(3) Hazard assessment at present and future courses of action

As described above, no obvious effect of di-2-ethylhexyl phthalate was observed in this study. With respect to testicular eggs observed at a low frequency without a dose-response relationship, the effect on population will be studied and then, the implementation of an additional study such as a full life cycle test should be considered.

5. Diethyl phthalate

(1) Study with medaka

1) Vitellogenin assay

This assay was conducted with this substance at an exposure concentration of 8.1, 26.8, 119.8, 355.8 and 1,053.3 μg/L (actual measured concentrations). A low value with statistical significance for the vitellogenin concentration in the liver was observed in males of the 1,053.3 μg/L group.

A low value with statistical significance for hepatosomatic index was observed in males of the groups exposed at a concentration of 8.1 μg/L or more at 14 days after exposure.

2) Partial life cycle test

This test was conducted with this substance at an exposure concentration of 0.6, 2.5, 8.4, 36.0 and 121.6 μg/L (actual measured concentrations). No statistically significant change in the hatchability, mortality, histological finding of genital gland, or the gonad index and hepatosomatic index was observed.

A statistically significant increase in the number of days until hatching was observed in the 2.5, 8.4 and 36.0 μg/L groups, and low values with statistical significance for the total length and body weight was observed in the 36.0 μg/L group. A statistically significant low body weight value was observed in the 121.6 μg/L group.

A low value with statistical significance for the vitellogenin concentration in the liver was observed in males of the121.6 μg/L group.

(2) *In vitro* study

A medaka estrogen receptor (ER α and ER β)-competitive binding assay, medaka estrogen receptor-reporter gene assay (ER α and ER β) and medaka androgen receptor-reporter gene assay were conducted.

As a result, the medaka estrogen receptor-reporter gene assay (ER β) showed the activity with respect to ER β but did not provide IC_{50} value. No statistically significant activity was shown in the medaka estrogen receptor (ER α and ER β)-competitive binding assay, medaka estrogen receptor-reporter gene assay (ER α) and medaka androgen receptor-reporter gene assay.

(3) Hazard assessment at present and future courses of action

As described above, the general toxicity (a statistically significant increase in the number of days until hatching, low value with statistical significance for the hepatosomatic index, and low values with statistical significance for the total length and body weight) of diethyl phthalate was observed at a relatively high dose in this study.

This study result will be used for the initial environmental risk assessment of this substance in the future.

7. Di-2-etylhexyl adipate

(1) Study with medaka

1) Vitellogenin assay

No statistically significant effect of this substance on the vitellogenin concentration in the liver was observed in males exposed at a concentration of 2.4, 7.9, 21.5, 181.7 and 453.6 μg/L (actual measured concentrations).

A low value with statistical significance for the hepatosomatic index was observed in males of the 2.4 μg/L dose group at 21 days after exposure.

2) Partial life cycle test

This test was conducted with this substance at an exposure concentration of 0.711, 2.33, 7.88, 26.3 and 87.1 μg/L (actual measured concentrations). No statistically significant change in the hatchability, the number of days until hatching, mortality, total length, vitellogenin concentration in the liver, the gonad index or the hepatosomatic index was observed. A statistically significant high body weight value was observed in the 7.88 μg/L group.

A histological test of the genital gland revealed a few fish having testicular eggs in the synergistic-substance control group and 7.88 μg/L group.

(2) *In vitro* study

A medaka estrogen receptor (ERα and ERβ)-competitive binding assay, medaka estrogen receptor-reporter gene assay (ERα and ERβ) and medaka androgen receptor-reporter gene assay were conducted.

As a result, the medaka estrogen receptor-competitive binding assay (ERβ) showed that the relative binding strength was about 1/2, 440 compared with estradiol. No statistically significant activity was shown in the medaka estrogen receptor-competitive binding assay (ERα), medaka estrogen receptor-reporter gene assay (ERα and ERβ) and medaka androgen receptor-reporter gene assay.

(3) Hazard assessment at present and future courses of action

As described above, the general toxicity (a low value with statistical significance for hepatosomatic index) of di-2-etylhexyl adipate was observed in this study. With respect to testicular eggs observed at a low frequency without a dose-response relationship, the effect on population will be studied and then, the implementation of an additional study such as a full life cycle test should be considered.

The results of reliability assessment of the literature on the adverse effects

1. DEHP

Of the five papers on the adverse effect of DEHP on the reproductive organs of boys, which was suggested to be evidence of "serious concern" by the Center for Evaluation of Risks to Human Reproduction (CERHR), four papers were subject to the reliability assessment. The fifth paper was a closed document, so that only the abstract of the lecture was available and detailed information was unknown.

Poon et al. have studied the effect of di-2-ethylhexyl phthalate on SD rats that were given a diet containing this substance at a concentration of 5, 50, 500 and 5,000 ppm for 13 weeks. As a result, there was no effect on weight gain, food consumption or morphological changes in the female ovary, however a slight vacuolation of Sertoli cells in the testis was observed in seven of ten rats of the 500 ppm dose group. Moreover, an atrophy of seminiferous tubules accompanied by a complete failure of spermatogenesis as well as a slight change in Sertoli cells was observed in nine of ten rats of the 5,000 ppm dose group. In the 5,000 ppm dose group, an increase in the liver weight was also observed. In addition, electron microscopy revealed hypertrophy and necrosis of hepatic cells with the proliferation of peroxisome and lysosome containing electron-dense lipid (in both male and female rats). An increase in relative kidney weight (female rats) and the effects on the hematological and serum biochemical analyses and the drug metabolizing enzyme activity (in both male and female rats) were also observed. Based on these changes in the testis, it was judged that the no-observed-adverse-effect level (NOAEL) was 500 ppm in a diet and 3.7 mg/kg/day as intake. The reliability of this test result was confirmed based on the literature.

Lamb et al. have studied the effect of di-2-ethylhexyl phthalate on CD-1 mice that were given a diet containing this substance at a concentration of 0.01, 0.1 and 0.3% for 98 days. As a result, there was no adverse effect on reproduction in the 0.01% dose group, however significant decreases in the frequency of parturition, the litter size and the live birth rate were observed in the 0.1% dose group. All of the mice in the 0.3% dose group were infertile. In the cross mating study, when the male mice of the 0.3% dose group were mated with female mice of the control group, decreases in the pregnancy rate and the litter size were observed, and when female mice of the 0.3% dose group were mated with the male mice of the control group, no mice became pregnant. Significantly decreases in the weight of testis, epididymis and prostate gland, significant decreases in the number of motile spermatozoa and concentrations of spermatozoa, and significant increases in the rate of abnormal spermatozoa were observed in the male mice of the 0.3% dose group. A decrease in the uterine weight was observed in the female mice, and an increase in the liver weight was also observed in both the male and female mice. From these results, it was judged that the no-observed-adverse-effect level (NOAEL) was

0.01% in a diet and 14 mg/kg/day as an intake. The reliability of this test result was confirmed based on the literature.

Gray et al. have studied the effect of diethylhexyl phthalate on male suckling rats from SD rats administered with this substance by oral gavage at a dose of 750 mg/kg/day from the 14th day of pregnancy to the third day of lactation. As a result, atrophy or aplasia of the testis and epididymis, and bleeding from the testis in the mice aged eight days were observed. The reliability of this test result was confirmed based on the literature.

Arcadi et al. have studied the effect of bis-di-2-ethylhexyl phthalate on the juvenile rats from Long-Evans rats given water containing this substance at a dose of 32.5 or 325 μL/L/day (it was estimated that the doses during pregnancy corresponded to 3.0-3.5 or 30-35 mg/kg/day and those during lactation corresponded to 30% in excess of these doses) from the first day of pregnancy to the 21st day after parturition . As a result, a significant decrease in the weight of testis correlated with the doses, delayed spermatogenesis up to eight-weeks after birth, a decrease in the kidney weight and abnormal histopathological findings of the kidney (atrophy of glomerulus accompanied by glomerular nephritis, dilation of the tubules in the renal medulla with epithelial cleavage, and mild fibrosis), liver (subendothelial edema of the lobular central vein and portal vein, and mild cellular infiltration) and the testis (major abnormal arrangement of the seminiferous tubules, deciduation of the spermatogonia from the basal membrane and aplasia of spermatocytes) were observed. In addition, a significant prolongation of the time required for rats to reach the goal was observed in female juvenile rats aged 30 days of the 325 μL/L/day dose group in avoidance learning with light/sound stimuli. In this study, water consumption and food consumption during the period when the dams were administered with this substance in water were not determined and spontaneous eating and drinking of juvenile rats were not observed. Therefore, it could not be confirmed whether or not the changes observed in the juvenile rats and the young rats were caused by the fetal exposure or the exposure via milk to this substance. The reliability was low based on the literature.

The following is shown from these reliable papers:

- With respect to the effect of di-2-ethylhexyl phthalate on testis, the reliable report said that a slight vacuolation of Sertoli cells and atrophy of seminiferous tubules with a complete failure of spermatogenesis were observed in the 13-week animal experiment, and it was judged based on the changes in testis that the no-observed-adverse-effect level (NOAEL) was 3.7 mg/kg/day. In the 98-day animal experiment, there was a reliable report that decreases in the weight of epididymis and prostate gland, decreases in the number of motile spermatozoa and the concentrations of spermatozoa, and an increase in the rate of abnormal spermatozoa were significantly observed. Further, in the animal experiment in which the effect on male suckling rats from dams administered with this substance from the 14th day of pregnancy to the 3rd day of lactation was studied, there was a reliable report that the atrophy or aplasia of testis and epididymis, and the bleeding from the testis

in the rats aged eight days were observed.

- With respect to the effect on reproduction, the reliable report said that significant decreases in the frequency of parturition, the litter size, the live birth rate and the uterine weight, and infertility were observed in the 98-day animal experiment, and it was judged that NOAEL was 14 mg/kg/day.
- There was a reliable report that an increase in the liver weight, hypertrophy and necrosis of hepatic cells with the proliferation of peroxisome and lysosome containing electron-dense lipid revealed by electron microscopy, an increase in relative kidney weight and the effects on hematological and serum biochemical test results and the drug metabolizing enzyme activity were observed.

This substance will be added to the list of the subjects given the high priority of risk assessment in 2000, since the effects on an endocrine organ (testis) and reproduction were observed in the animal experiments.

3. Di-ethyl phthalate

With respect to the adverse effects of di-ethyl phthalate, there are some reports in the existing literature on the presence or absence of estrogenic activity and the presence or absence of the effect related to the reproductive toxicity. The reliability of each paper showing these effects was assessed, and the conclusion was summarized as follows from the perspective of whether or not this substance should currently be a subject for risk assessment.

(1) Estrogenic activity

Harris et al. have conducted a recombinant yeast assay with di-ethyl phthalate and a study on the effect of this substance on the proliferation of human breast cancer cell lines, ZR-75 and MCF-7. In the recombinant yeast assay, a positive result was obtained by di-ethyl phthalate at a high concentration of 10^{-4}-10^{-3} M, however the activity was as low as 5/10,000,000 that of 17β-estoradiol. Further, the division and growth of ZR-75 cells and MCF-7 cells were not promoted by this substance at a concentration of 10^{-7}-10^{-5}M and 10^{-5}M, respectively. These results suggested that this substance had weak estrogenic activity but this result could not be directly extrapolated to determine the effect on humans. The reliability of this result was confirmed based on the literature.

Lutz et al. have studied the binding-inhibitory effect of di-ethyl phthalate on hepatic estrogen receptors in Xenopus. Di-ethyl phthalate at a high concentration of 10^{-3} M was entirely competed with 17-β estoradiol to bind with estrogen receptors, and the IC_{50} value was 12×10^{-6}M. The reliability of this result was confirmed based on the literature.

(2) Reproductive toxicity

Lamb et al. have studied the effect of di-ethyl phthalate on male and female COBS Crl: CD-1(ICR) mice aged six weeks that were given a diet containing this substance at a concentration of 0.25, 1.25 and 2.5% from seven days before mating for 98 days. As a result, a body weight loss of the parent mice, a decrease in the weight of the prostate gland and a decrease in the concentration of spermatozoa in the sires, a decrease in the weight of the liver and pituitary and a decrease in the live litter size in the dams were observed. The reliability of this result was confirmed based on the literature.

Field et al. have studied the effect of di-ethyl phthalate on female Crl: CD (SD) BR VAF/Plus SD rats that were given a diet containing this substance at a dose of 200, 1,910 and 3,210 mg/kg/day from the 6th to 15th day of pregnancy. As a result, an inhibition against weight gain was observed in the dams of the groups administered with this substance at a dose of 1,910 mg/kg/day or higher. An increase in the relative liver weight to the body weight in the dams and the occurrence of supernumerary rib in the fetuses were observed in the 3,210 mg/kg/day dose group. These results suggested that the no-observed-adverse-effect level of maternal toxicity was 200 mg/kg/day and that of developmental toxicity was 1,910 mg/kg/day. The reliability of this result was confirmed based on the literature.

Oishi et al. have studied the effect of di-ethyl phthalate on male Wistar rats given a diet containing 2% of this substance for one week. As a result, a decrease in testosterone concentrations in testis and serum was observed. The reliability of this result was confirmed based on the literature.

Singh et al. have studied the effect of di-ethyl phthalate on SD rats that were intraperitoneally administered with this substance at a dose of 500, 1,000 and 1,500 mg/kg on the 5th, 10th and 15th day of pregnancy . As a result, a fetal body weight loss and fetal skeleton malformation were observed in the groups administered with this substance at a dose of 500 mg/kg or higher. The reliability of this result was confirmed based on the literature.

The following is shown from the literature currently available:

- With respect to the estrogenic activity of di-ethyl phthalate in the *in-vitro* studies, there was a reliable report that weak estrogenic activity was observed in the recombinant yeast assay, however no cell proliferation was observed in the cell-proliferation study. In addition, the reliable report said that the binding inhibition to estrogen receptors was observed.
- In the animal experiments, there was a reliable report that a body weight loss, a decrease in the weight of the prostate gland, a decrease in the concentration of spermatozoa, a decrease in the concentration of testosterone, a decrease in the weight of the liver and pituitary, a decrease in the live litter size, a fetal body weight loss, and occurrences of supernumerary rib and skeleton malformation were observed.

Di-ethyl phthalate is to be a subject for risk assessment, since the effect on the prostate gland and spermatozoa, a decrease in the concentration of testosterone, the effect on an endocrine organ such as pituitary and a effect on fetuses have been observed.

5. Di-etylhexyl adipate

Reliability assessment results of the literature on the adverse effect of di-2-etylhexyl adipate

With respect to the adverse effects of di-2-etylhexyl adipate, there are some reports in the existing literature on the presence or absence of estrogenic activity and the presence or absence of the effect related to the reproductive toxicity. The reliability of each paper showing these effects was assessed, and the results were summarized as follows from the perspective of whether or not this substance should be a subject for risk assessment at present.

(1) Estrogenic activity

Jobling et al. have studied the inhibitory effect of di-2-etylhexyl adipate on the binding of 17β-estoradiol to hepatic estrogen receptors in rainbow trout and the effect on the proliferation of human breast cancer cells, ZR-75. The binding of 17 β -estoradiol to estrogen receptors was inhibited by di-2-etylhexyl adipate at a concentration of 10^{-6}-10^{-3} M, however the division and growth of ZR-75 cells were not promoted by this substance at a concentration of 10^{-5} M. Thus, it has not been suggested that this substance has estrogenic activity. The reliability of this report was confirmed based on the literature in general.

(2) Reproductive toxicity

Singh et al. have studied the effect of di-2-etylhexyl adipate on Harlan/ICR albino Swiss mice aged 8-10 weeks, of which the male mice were intraperitoneally administered with this substance once at a dose of 0.5, 1, 5 and 10 ml/kg and then mated with non-treated females. In addition, they have studied the effect of this substance on fetuses from female SD rats which were intraperitoneally administered with this substance at a dose of 1, 5 and 10 ml/kg/day on the 5th, 10th and 15th day of pregnancy. As a result, a decrease in the pregnancy rate and an increase in the number of early fetal deaths with a dose-response relationship were observed in female mice mated with the male mice of the 10 mg/kg dose group, suggesting male sterility. A fetal body weight loss and an increase in malformation were observed in rats of groups administered with this substance at a dose of 5 ml/kg/day or higher, and the no-observed-adverse-effect level was considered to be 1 ml/kg/day. The reliability of these results was confirmed based on the literature.

The following is shown from the literatures currently available:

- With respect to the estrogenic activity of di-2-etylhexyl adipate, there was a reliable report that this substance was bound to hepatic estrogen receptors in rainbow trout in the *in-vitro* study, however no cell proliferation was observed in the subsequent cell-proliferation study.
- In the animal experiments, the reliable report said that a decrease in the pregnancy rate, male infertility and the effect on fetuses were observed.

Di-2-etylhexyl adipate is to be a subject for risk assessment, since pregnancy failure and the effect on fetuses was recognized in the animal experiments. With respect to the estrogenic activity, further *in-vitro* study should be conducted to reconfirm the presence or absence of the activity.